A Putative Exonic Splicing Polymorphism in the BCL6 Gene and the Risk of Non-Hodgkin Lymphoma

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Recent studies have shown that the B-cell lymphoma 6 gene (BCL6) is an oncogene that contributes to lymphomagenesis. Exon 6 of BCL6 contains a common single nucleotide polymorphism (SNP) (−195 C>T; dbSNP ID: rs1056932) that alters a potential binding site for an exonic splicing enhancer. We used unconditional logistic regression models to examine the association between this SNP and the risk of non-Hodgkin lymphoma (NHL) in a population-based case–control study of women residing in Connecticut (461 case patients and 535 control subjects). The risk of NHL among women with the CC genotype was more than double that of women with the TT genotype (odds ratio [OR] = 2.2, 95% confidence interval [CI] = 1.5 to 3.3). Higher risks were observed for two NHL subtypes, namely B-cell chronic lymphatic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma (OR = 3.5, 95% CI = 1.6 to 7.8) and T-cell lymphoma (OR = 5.2, 95% CI = 2.0 to 13.3). Our results support the hypothesis that a genetic variant that could alter mRNA transcripts of BCL6 may contribute to the etiology of NHL and suggest that this variant warrants further investigation. [J Natl Cancer Inst 2005;97:1616–8]

The B-cell lymphoma 6 gene (BCL6) was discovered during an analysis of chromosome 3q27 translocations in diffuse large B-cell lymphomas and follicular lymphomas *(1–3)*. BCL6 encodes the POZ/zinc finger transcriptional factor Bcl6, which is necessary for germinal center formation and for the T-cell-dependent antibody response *(4)*. BCL6 is expressed early in germinal center formation, but its expression is reduced in post-germinal center cells, suggesting that Bcl6 plays a critical role in lymphopoeisis, one that, if disturbed, could contribute to lymphomagenesis *(5)*.

It has been shown that Bcl6 represses expression of a number of genes that are functionally linked to B-cell activation and differentiation (i.e., CD44, CD69, STAT1, LEU13, Blimp-1), inflammation (i.e., IP10, CXCR4), and cell cycle control (i.e., p27kip1, cyclin D2) *(6)*. Deregulated expression of BCL6 could alter the expression or function of genes in these critical pathways, resulting in the promotion of tumorigenesis. Results of a recent study in transgenic mice suggest that BCL6 can act as an oncogene in lymphomagenesis *(7)*.

In view of the seminal importance of BCL6 in lymphogenesis, we examined the association between a potentially functional single nucleotide polymorphism (SNP) in BCL6 exon 6 (Ex6−195C>T; dbSNP ID: rs1056932) that could alter BCL6 mRNA expression and the risk of non-Hodgkin lymphoma (NHL) in a large population-based case–control study among women residing in Connecticut.

A detailed description of the study population has been reported previously *(8–12)*. A total of 601 case patients with histologically confirmed incident NHL and 717 population-based control subjects were enrolled and completed in-person interviews. Blood samples for genotyping were available for 461 (76.7%) case patients and 535 (74.6%) control subjects. DNA was extracted from the blood samples using phenol-chloroform extraction *(13)* and genotyped by a real-time polymerase chain reaction assay on an ABI 7900HT sequence detection system as described on the SNP500 website (http://snp500cancer.nci.nih.gov/snp.cfm?both_snp_id=BCL6-01) at the Core Genotyping Facility of the National Cancer Institute *(14)*. The study was approved by Institutional Review Boards at Yale University, the Connecticut Department of Public Health, and the National Cancer Institute. Participants provided written informed consent.

Unconditional logistic regression models were used to estimate the association between the genetic polymorphism in the BCL6 gene and the risk of NHL by NHL subtypes. In the model, we assigned the TT, CT, and CC genotypes values of 0, 1, and 2, respectively, and treated genotype as a continuous variable for trend analyses. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using SAS software (version 8.02; SAS Institute, Inc., Cary, NC). All statistical tests were two-sided.

A greater proportion of NHL case patients than control subjects reported having a family history of NHL and other cancers among their first-degree relatives (Table 1). There were no statistically significant differences in age or race between case patients and control subjects.

We found that the homozygous C allele of the BCL6 Ex6−195C>T SNP was associated with an increased risk of NHL (Table 2). Compared with the TT genotype, the CC genotype was associated with statistically significantly increased risks of NHL overall (OR = 2.2, 95% CI = 1.5 to 3.3), B-cell lymphoma (OR = 2.0, 95% CI = 1.3 to 3.1), and T-cell lymphoma (OR = 5.2, 95% CI = 2.0 to13.3). The association between the C allele and risk was dose dependent (P_{trend} = .0002, .0018, and .0016, respectively).

We also found that the homozygous C allele was associated with statistically significantly increased risks of three subtypes of B-cell lymphoma: B-cell chronic lymphocytic leukemia/prolymphocytic

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Table 1. Selected characteristics of study participants who provided blood samples for genotyping*

Characteristic	Case patients (n = 461) n (%)	Control subjects (n = 535) n (%)	P†
Age, y			
<40	36 (7.8)	45 (8.4)	.40
40–49	55 (11.9)	54 (10.1)	
50–59	96 (20.8)	99 (18.5)	
60–69	117 (25.4)	125 (23.4)	
≥70	157 (34.1)	212 (39.6)	
Race			
White	444 (96.3)	504 (94.2)	.06
Non-Hispanic	437 (94.8)	491 (91.8)	
Hispanic	7 (1.5)	13 (2.4)	
African-American	13 (2.8)	14 (2.6)	
Other	4 (0.9)	17 (3.2)	
Family history‡			
No	97 (21.0)	131 (24.5)	.03
NHL	9 (2.0)	2 (0.4)	
Other cancer	355 (77.0)	402 (75.1)	
Pathology			
All B cell	369 (80.0)		
Diffuse large B-cell	147 (31.9)		
Follicular	106 (23.0)		
CLL/SLL	54 (11.7)		
Marginal zone	31 (6.7)		
Other§	31 (6.7)		
All T cell	33 (7.2)		
NOS	59 (12.8)		

*NHL = non-Hodgkin lymphoma; CLL/SLL = B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma; NOS = not otherwise specified.

†Chi-square test (two-sided).

‡Family history of cancer in first-degree relatives.

§Includes mantle cell lymphoma (n = 12), plasmacytoma (n = 1), Burkitt's lymphoma (n = 1), and unspecified B-cell lymphoma (n = 17).

leukemia/small lymphocytic lymphoma (CLL/SLL; OR = 3.5, 95% CI = 1.6 to 7.8), diffuse large B-cell lymphoma (OR = 2.0, 95% CI = 1.1 to 3.5), and follicular lymphoma (OR = 2.0, 95% CI = 1.1 to 3.8). Statistically significant trends were observed for associations between the variant genotypes and the risks of CLL/SLL and diffuse large B-cell lymphoma (P_{trend} = .0029 and .016, respectively), but not between the variant genotypes and the risk of follicular lymphoma (P_{trend} = .11). Further analyses that were restricted to non-Hispanic white subjects produced results similar to those reported above (data not shown).

Our results, which are the first evidence of an association between a BCL6 SNP and NHL from a population-based molecular epidemiology study, support the hypothesis that a common genetic variation in the BCL6 gene could contribute to lymphomagenesis. It has been reported that this same SNP is associated with an increased risk of breast cancer *(15)*. It is interesting to note that, in our study, the BCL6 Ex6−195C>T SNP was associated with multiple histologic subtypes of NHL. Although the exact mechanism(s) underlying such a phenomenon is currently unknown, this finding suggests that multiple lymphoma subtypes may share an early step in lymphomagenesis.

The synonymous Ex6−195C>T SNP, which does not change the amino acid sequence of Bcl6, is a particularly interesting genetic variant because it can potentially alter the pattern of mRNA splicing involving one of the functional domains within the BCL6 gene. We used the web-based tool ESEfinder (http://exon.cshl.edu/ESE/) to examine the effects of this genetic variant on splicing and found that the Ex6–195C>T SNP could potentially cause changes of these binding sites for exonic splicing enhancers that could lead to the inaccurate recognition of exon/intron boundaries or differential binding of transcription

Table 2. Association between a single nucleotide polymorphism in the BCL6 gene and NHL*

		NHL											
		All NHL			B-cell lymphoma			T-cell lymphoma					
Genotype	No. of control subjects	No. of case patients	OR (95% CI)	P	No. of case patients	OR (95% CI)	P	No. of case patients	OR (95% CI)	P	No. of case patients	OR (95% CI)	P
TT	235	166	1.0 (referent)		137	1.0 (referent)		9	1.0 (referent)				
CT	237	210	1.3 (1.0 to 1.7)	.081	167	1.2 (0.9 to 1.7)	.14	13	1.4 (0.6 to 3.4)	.42			
CC	58	81	2.2 (1.5 to 3.3)	.0001	62	2.0 (1.3 to 3.1)	.0012	11	5.2 (2.0 to 13.3)	.0006			
P_{trend}			.0002			.0018			.0016				
		B-cell lymphoma subtype											
		CLL/SLL			DLBL			MZBL			FL		
TT	235	15	1.0 (referent)		52	1.0 (referent)		14	1.0 (referent)		44	1.0 (referent)	
CT	237	26	1.7 (0.9 to 3.3)	.11	72	1.4 (0.9 to 2.1)	.10	11	0.8 (0.4 to 1.9)	.67	41	0.9 (0.6 to 1.5)	.81
CC	58	13	3.5 (1.6 to 7.8)	.0024	23	2.0 (1.1 to 3.5)	.023	5	1.6 (0.6 to 4.8)	.37	19	2.0 (1.1 to 3.8)	.027
P_{trend}			.0029			.016			.61			.11	

*The SNP genotype frequency in the control subjects was in Hardy-Weinberg equilibrium. Odds ratios were adjusted for age (as a continuous variable), race, and family history of NHL in first-degree relatives. *P* values are two-sided (Wald chi-square test). NHL = non-Hodgkin lymphoma; OR = odds ratio; CI =confidence interval; CLL/SLL= B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma; DLBL = diffuse large B-cell lymphoma; MZBL = marginal zone B-cell lymphoma; FL = follicular lymphoma. Four case patients and five control subjects were excluded from the analysis because their genotypes could not be determined.

factors to the BCL6 pre-mRNA. A functional domain in the center of the BCL6 protein that could act as an autonomous transcriptional repression module by recruiting histone deacetylases has been characterized and mapped to a 17-amino acid sequence *(16)*. The SNP investigated in our study is located immediately carboxyl-terminal to this 17-amino acid sequence and could alter the binding site for exonic splicing enhancers (ESEs), which are short binding sites for serine/arginine-rich proteins involved in multiple steps of the splicing pathway *(17)*.

However, it is also possible that the Ex6−195C>T SNP is in linkage disequilibrium with another variant located in or near the BCL6 gene that is associated with an increased risk of NHL. Additional studies are needed to survey the common genetic variants across this locus, to determine the pattern of linkage disequilibrium, and to characterize the common haplotype structure. It is also possible that one or more additional SNPs, in *cis*, could be associated with an increased risk of NHL. Thus, additional studies should be performed in parallel with the genetic studies to investigate the functional consequences of the Ex6−195C>T SNP and, perhaps, of other SNPs in linkage with it.

One potential limitation of this study is that blood samples for genotype analysis were not available for all study subjects. However, we found no differences between subjects with and without blood samples in terms of age, race, family history of NHL in first-degree relatives, or case–control status. Another potential concern is that a small number of case patients who had very aggressive disease may have died before the interview and thus were underrepresented in this study. Therefore, our results may not be generalizable to individuals with aggressive forms of NHL. Similarly, because our study included only women, our results may not be generalizable to men.

In summary, our study provides the first evidence that genetic variation in the coding region of the BCL6 gene is associated with inherited susceptibility to NHL. Large molecular epidemiology studies should be conducted to investigate the impact of this type of SNP on the disruption of a putative splicing site in different study populations.

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Notes

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